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DISTRIBUTION OF ELLAGIC ACID DERIVATIVES AND A DIARYLHEPTANOID IN WOOD OF *PLATYCARYA STROBILACEA*

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Key Word Index—*Platycarya strobilacea*; Juglandaceae; wood; ellagic acid; diarylheptanoid.

Abstract—4-*O*-Xylosides of ellagic acid, 3′-*O*-methylellagic acid and 3,3′-di-*O*-methylellagic acid have been isolated from the sapwood of *Platycarya strobilacea*; these compounds were absent in the heartwood. A new diphenylether-type diarylheptanoid, named platycarynol, was also isolated from the heartwood, together with ellagic acid, 3′-*O*-methylellagic acid and two known terpenoids. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Recently we have reported on the presence of whiskylactone (quercuslactone) precursors (**1** and its desgalloyl derivative) and hydrolysable tannins in the wood of *Platycarya strobilacea* Sieb. et Zucc. [1]. The concentration of the whiskylactone precursors was higher in the heartwood than sapwood, and hydrolysable tannins were found only in the heartwood. In the course of our studies on the metabolism of polyphenolic compounds in wood [2], we now report the distribution of ellagic acid derivatives in the wood of *P. strobilacea* and the structural determination of a new diarylheptanoid, which is located only in the heartwood.

RESULTS AND DISCUSSION

From the aqueous acetone extract of fresh wood, we isolated eight compounds (**1–8**), together with gallic acid, by repeated chromatography over Sephadex LH-20, MCI-gel CHP20P, Chromatorex ODS and silica gel. Compounds **2** and **4–8** were identified as ellagic acid 4-*O*-xylopyranoside [3], 3,3′-di-*O*-methylellagic acid 4-*O*-xylpyranoside [4], ellagic acid, 3-*O*-methylellagic acid [5], 3-cadalenol [6] and (−)-(7*S*,10*R*)-3-hydroxycalamenene [7], respectively, by comparison of their spectroscopic data with those of authentic samples or values reported in the literature.

Compound **3** was obtained as colourless needles and found to be a new compound. The ^{1}H and ^{13}C NMR spectra were closely related to those of **2** and **4**, showing the presence of a xylopyranose moiety. Appearance of methoxyl signals (δ 4.01) in the ^{1}H NMR spectrum and an $[M-H]^-$ ion peak at *m/z* 447 in the negative ion FAB mass spectrum indicated that **3** is a monomethyl derivative of **2**. The chemical shift of the methyl carbon (δ 60.8) was similar to that of the 3-*O*-methylderivative (δ 60.9, **6**) but different from that of the 4-*O*-methylderivative (δ 56–57, [3]), suggesting location of the methoxyl group at C-3 or C-3′. This was confirmed by a two-dimensional NOESY experiment of the pentaacetate (**3a**), in which one (δ 2.40) of the acetyl signals showed a cross-peak with both of the methoxyl signals (δ 4.29) and one (δ 7.89) of the aromatic protons. This result indicated location of the methoxyl group at C-3′ and the acetyl group at C-4′. These observations indicated unequivocally that compound **3** is 3′-*O*-methylellagic acid 4-*O*-xylopyranoside.

Compound **9**, named platycarynol, was obtained as colourless needles and showed an $[M]^+$ at *m/z* 342, suggesting the molecular formula $C_{21}H_{26}O_4$. The ^{1}H NMR spectrum showed signals attributable to two tri-substituted aromatic rings, two aromatic methoxyl groups, six methylenes and one oxygen bearing methine, suggesting that **9** is a diarylheptanoid having two methoxyl groups and one secondary alcohol. Complete assignments of these proton signals were achieved by COSY and NOESY spectral analyses. Appearance of an aromatic proton signal (H-6) at unusually high field (δ 5.69) is characteristic of a diphenylether-type diarylheptanoid [8]; NOESY correlation between this proton and an aromatic proton (H-18) of the other aromatic ring confirmed its macrocyclic structure. The location of the two methoxyl groups at C-2 and C-16 was determined by observation of NOE correlation between one of the methoxyl groups (δ 3.95) and H-3 and between the other (δ 3.71) and H-15. Finally, the absolute configuration

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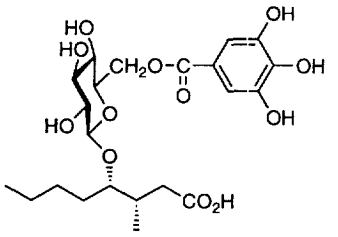

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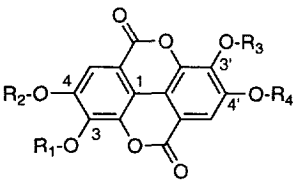
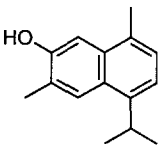


	R_1	R_2	R_3	R_4
2	H	xylose	H	H
3	H	xylose	CH_3	H
3a	Ac	xylose(triAc)	CH_3	Ac
4	CH_3	xylose	CH_3	H
5	H	H	H	H
6	CH_3	H	H	H



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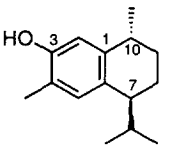

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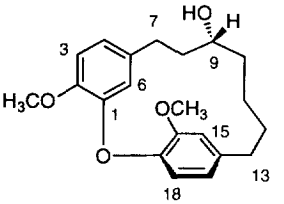

9

of the aliphatic methine (C-9) was established to be *R* by application of the modified Mosher's method [9] to (*R*)- and (*S*)-MTPA esters of **9**. Thus, the structure of this new diarylheptanoid was represented by the formula **9**. This compound is the first diarylheptanoid isolated from the Juglandaceae.

HPLC analysis (UV 280 nm) of the wood showed the presence of whiskylactone precursor (**1**) in both sapwood and heartwood, and its concentration linearly increased with distance from the cambium [1]. On the other hand, compounds **2** and **3** which are accumulated in the sapwood completely disappeared in the heartwood, whereas a steep increase in the concentration of compounds **5** and **6** was observed in the heartwood (Fig. 1). It is known that sapwood contains living parenchymal cells but all cells are dead in heartwood [10]. As the tree grows, the parenchymal cells of the inner sapwood die and the heartwood extends. This strongly suggests that the glycosides **2** and **3** are precursors of **5** and **6**, respectively. The large increase of ellagic acid (**5**) may also be accounted for by hydrolysis of ellagitannins [1] or oxidative coupling of gallic acid which was also accumulated in the heartwood. Furthermore, TLC comparison revealed the presence of the three non-polar compounds **7**–**9** only in the heartwood.

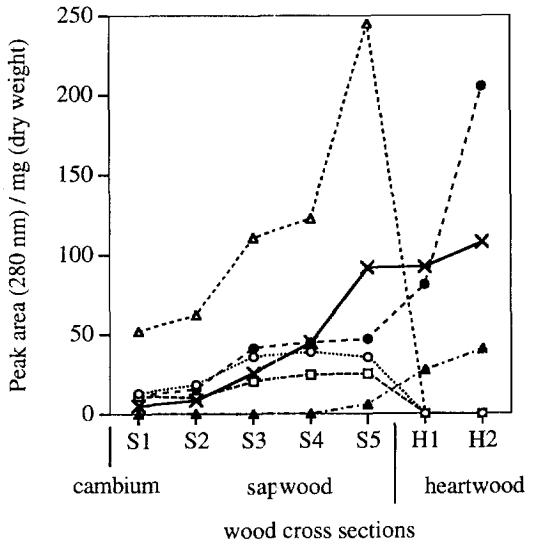

Fig. 1. Distribution of tannins in wood cross-sections of *Platycarya strobilacea*. S, sapwood; H, heartwood; S1: cambium; H2: centre of wood; (×) 1; (○) 2; (△) 3; (□) 4; (●) 5; (▲) 6.

EXPERIMENTAL

General

^1H and ^{13}C NMR spectra were recorded at 300 and 500 MHz, and 75 and 125 MHz, respectively. Chemical shifts are given in δ with TMS as int. standard. Negative FABMS was measured at 1.5 kV with MeOH-glycerol as matrix, EIMS at 30 eV. CC was carried out on Sephadex LH-20 (20–100 μm, Pharmacia), MCI-gel CHP20P (Mitsubishi Chemical Co.), TSK-gel Toyopearl HW40F(TOSOH) Avicel cellulose (Funakoshi), Bondapak C_{18}/Porasil B (Waters), Kiesel gel 60 (Merck), Cosmosil 75C18-OPN (Nacalai tesque) and Chromatorex ODS (Fuji Silysia). TLC was conducted on precoated silica gel 60 F_{254} plates and precoated cellulose F_{254} plates; spots were detected under UV and by spraying ethanolic $FeCl_3$ reagent (for phenolics) and dil. H_2SO_4, followed by heating (for Me ethers). Analytical HPLC was performed on a Cosmosil $5C_{18}$-AR (Nacalai Tesque Inc.) column (4.6 mm i.d. × 250 mm) (mobile phase, MeCN-50 mM H_3PO_4, gradient elution from 5 to 35% MeCN for 30 min; flow rate, 0.8 ml^{-1} min; UV detection at 280 nm).

HPLC analysis

Samples (100–200 mg) cut out of sapwood (5 pieces) and heartwood (2 pieces) were dried and separately

extracted ×2 with 70% aq. Me_2CO (3 ml). After concn, each extract was passed through a Sep-pak C_{18} cartridge (Waters) with 50% MeOH (3 ml) and analyzed by reverse-phase HPLC. Peak areas were obtained using an integrator connected to a UV detector.

Isolation

Fr. wood (1.7 kg) was chipped into small pieces and extracted with 70% aq. Me_2CO. After evapn of solvent, insol. ppts were removed by filtration and the filtrate was successively extracted with Et_2O and EtOAc. The ppts were treated with MeOH and the sol. fr. subjected to silica gel CC with $CHCl_3$ to afford **9** (290 mg) and a mixt. of **7** and **8**, which was separated by silica gel CC with hexane–EtOAc (**7**, 128 mg and **8**, 89 mg). The Et_2O layer (1.2 g) was subjected to silica gel CC with benzene–Me_2CO to yield **9** (500 mg). The EtOAc layer (6.0 g) was subjected to MCI-gel CHP20 CC with H_2O–MeOH. Elution with 10–40% MeOH afforded gallic acid (234 mg), 1(*β*)-*O*-galloylpedunculagin (120 mg), eugeniin (30 mg), pentagalloylglucose (95 mg) and **1** (167 mg). The fr. obtained by elution of the column with MeOH was further separated by Sephadex LH-20 CC (80% MeOH) to yield **5** (280 mg) and **6** (59 mg). The aq. layer was concd and subjected to Sephadex LH-20 CC with H_2O–MeOH. The first fr. obtained by elution with H_2O was chromatographed over MCI gel CHP20P (H_2O–MeOH) to give 6 frs, 1–6. Fr. 5 (1.6 g) was concd and the resulting ppt. (420 mg) collected by filtration, which was separated by MCI-gel CHP20P CC (50–60% MeOH) to give **2** (30 mg) and **3** (70 mg). Fr. 6 (1.9 g) was successively subjected to CC over Prep pak 500/C_{18} (70–100% MeOH) and silica gel ($CHCl_3$–MeOH–H_2O, 70:30:5) to yield **4** (120 mg) and **3** (80 mg).

Ellagic acid 4-O-*xylopyranoside* (**2**)

Tan powder, mp 253–255° (decomp.). $[\alpha]_D^{20}$ −39.7° [C_5H_5N–DMSO (1:1); *c* 0.1]. ^1H NMR (DMSO-d_6): *δ* 3.30 (4H, *m*), 3.83 (1H, *dd*, *J* = 5, 11 Hz, xyl-5), 4.98 (1H, *d*, *J* = 7 Hz, xyl-1), 7.48 (1H, *s*, H-5′), 7.69 (1H, *s*, H-5). ^{13}C NMR (DMSO-d_6): *δ* 65.8 (xyl-5), 69.3 (xyl-4), 73.0 (xyl-2), 75.5 (xyl-3), 102.9 (xyl-1), 107.2, 108.1 110.3, 111.5, 111.7, 114.8, 136.1, 136.7, 139.7, 141.3, 146.9, 148.8, 158.9, 159.0.

3′-O-*Methylellagic acid* 4-O-*xylopyranoside* (**3**)

Colourless needles (H_2O–MeOH, 1:9), mp 218–219°. $[\alpha]_D^{28}$ −58.4° (C_5H_5N; *c* 0.3). Negative FABMS *m/z*: 447 $[M-H]^-$. ^1H NMR (DMSO-d_6): *δ* 3.20 (4H, *m*), 3.79 (1H, *dd*, *J* = 5, 11 Hz, xyl-5), 4.01 (3H, *s*, CH_3), 4.56 (1H, *d*, *J* = 7 Hz, xyl-1), 7.47, 7.48 (each 1H, *s*, H-5 and H-5′). ^{13}C NMR (DMSO-d_6): *δ* 159.9, 159.3 (COO), 152.3 (C-3′), 149.6 (2C), 142.4, 139.7, 137.5 (aromatic C-OH), 116.9, 115.7, 113.8, 112.4, 110.5 (2C) (aromatic CH), 105.1 (xyl-1), 76.3 (xyl-3), 73.2 (xyl-2), 69.3 (xyl-4), 66.1 (xyl-5), 60.8 (OMe). (Found: C, 47.66; H, 3.96; $C_{20}H_{16}O_{12}\cdot 3H_2O$ requires: C, 47.82; H, 4.41).

Acetylation of **3**

3 (30 mg) was acetylated with Ac_2O–C_5H_5N and purified by CC over silica gel (hexane–EtOAc) to give **3a** (26 mg) as a white powder. ^1H NMR ($CDCl_3$): *δ* 2.09, 2.09 and 2.10 (each 3H, *s*, xylose-OAc), 2.40 (3H, *s*, 4′-OAc), 2.44 (3H, *s*, 3-OAc), 3.69 (1H, *dd*, *J* = 8, 12 Hz, xylose H-5), 4.29 (3H, *s*, OMe), 4.30 (1H, *dd*, *J* = 5, 12 Hz, xylose H-5), 5.30 (3H, *brs*, xylose H-1, 2 and 3), 7.85 (1H, *s*, H-5), 7.89 (1H, *s*, H-5′).

3-O-*methylellagic acid* (**6**)

White powder, mp > 300°. ^1H NMR (DMSO-d_6): *δ* 4.04 (3H, *s*), 7.44, 7.49 (each 1H, *s*). ^{13}C NMR (DMSO-d_6): *δ* 60.9, 107.3, 110.3, 111.3, 111.8, 112.1, 112.4, 136.1, 139.7, 140.1, 141.4, 148.2, 152.1, 158.7, 158.8.

Platycarynol (**9**)

Colourless needles (hexane–EtOAc), mp 137–138°. $[\alpha]_D^{20}$ −17.1° ($CHCl_3$; *c* 0.7). Found: $[M]^+$ 342.1830; $C_{21}H_{26}O_4$ requires 342.1831. ^1H NMR ($CDCl_3$): *δ* 1.18 (1H, *brs*, C-9 OH), 0.88 (1H, *m*, H-10), 1.08 (1H, *m*, H-11), 1.24 (1H, *m*, H-10), 1.32 (1H, *m*, H-11), 1.49 (2H, *m*, H-8), 1.77, 1.54 (each 1H, *m*, H-12), 2.56 (1H, *ddd*, *J* = 3.4, 6.4, 16.7 Hz, H-7), 2.60 (1H, *ddd*, *J* = 5.0, 5.0, 13.0 Hz, H-13), 2.64 (1H, *ddd*, *J* = 3.7, 9.6, 16.7 Hz, H-7), 2.79 (1H, *ddd*, *J* = 4.3, 6.0, 13.0 Hz, H-13), 3.14 (1H, *brd*, *J* 7.1 Hz, H-9), 3.71 (3H, *s*, C_{16}-OCH_3), 3.95 (3H, *s*, C-2-OCH_3), 5.69 (1H, *d*, *J* = 2.1 Hz, H-6), 6.67 (1H, *dd*, *J* = 2.1, 8.0 Hz, H-4), 6.82 (1H, *d*, *J* = 8.0 Hz, H-3), 6.83 (1H, *d*, *J* = 2.1 Hz, H-15), 6.88 (1H, *dd*, *J* = 2.1, 8.0 Hz, H-19), 7.10 (1H, *d*, *J* = 8.0 Hz, H-18). ^{13}C NMR ($CDCl_3$): *δ* 22.7, 28.5, 30.3, 35.8, 36.9, 38.6 (C-7, C-8, C-10, C-11, C-12 and C-13), 56.4, 56.5 (OMe), 72.4 (C-9), 112.2, 113.3, 115.7, 121.7, 122.2, 124.5, 134.5, 140.8, 143.6, 146.4, 150.1, 152.3.

Preparation of MTPA esters of **9**

A soln of **9** (10 mg), dicyclohexylcarbodiimide (10 mg), 4-dimethylaminopyridine (5 mg) and (*R*)-(+)-α-methoxy-α-trifluoromethyl-phenylacetic acid (10 mg) in CH_2Cl_2 (1 ml) was left to stand at room temp. for 12 h. The resulting mixt. was separated by silica gel CC with hexane–EtOAc to give the (*R*)-MTPA ester (11 mg). The use of (*S*)-(−)-α-methoxy-α-trifluoromethyl-phenylacetic acid gave the (*s*)-MTPA ester (7 mg). $\Delta\delta$ (δ_S–δ_R): H-3 (−0.012), H-4 (−0.06), H-6 (−0.027), H-7 (−0.169, −0.113), H-8 (−0.086, −0.027), H-9 (−0.034), H-10 (+0.066, +0.111), H-11 (+0.033, +0.083), H-12 (+0.016, +0.043), H-13

(+0.027, −0.001), H-15 (+0.013), H-18 (+0.002), H-19 (+0.012), 2-OMe (+0.023), 16-OMe (0).

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REFERENCES

1. Tanaka, T. and Kouno, I., *J. Nat. Prod.*, 1996, **59**, 997.
2. Tanaka, T., Ueda, N., Shinohara, H., Nonaka, G., Fujioka, T., Mihashi, M. and Kouno, I., *Chem. Pharm. Bull.*, 1996, **44**, 2236.
3. Pei, Y. H., Li, X. and Zhu, T. R., *Yaoxue Xuebao*, 1991, **25**, 798 (CA 117:86659r).
4. Khac, D. D., Tran-Van, S., Campos, A. M., Lallemand, J. Y. and Fetizon, M., *Phytochemistry*, 1990, **29**, 251.
5. Hillis, W. E. and Yazaki, Y., *Phytochemistry*, 1973, **12**, 2963.
6. Alexander, J. and Rao, G. S. K., *Tetrahedron*, 1971, **27**, 645.
7. Nabeta, K., Katayama, K., Nakagawara, S. and Katoh, K., *Phytochemistry*, 1993, **32**, 117.
8. Nagumo, S., Ishizuka, S., Nagai, M. and Inouye, T., *Chem. Pharm. Bull.*, 1996, **44**, 1086.
9. Kusumi, T., Ohtani, I., Inoue, Y. and Kakisawa, H., *Tetrahedron Letters*, 1988, **29**, 4731.
10. Kemp, M. S. and Burden, R. S., *Phytochemistry*, 1986, **25**, 1261.